REFERENCE COUNT: 2 THERE ARE 2 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L22 ANSWER 2 OF 59 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 2001:862522 SCISEARCH
THE GENUINE ARTICLE: 486CN
TITLE: High **lipase** production by Candida **rugosa** is associated with G1 cells. A flow cytometry study
AUTHOR: Lotti M (Reprint); Brocca S; Porro D
CORPORATE SOURCE: Univ Milan, Dipartimento Biotecnol & Biosci, Piazza Sci 2, I-20126 Milan, Italy (Reprint); Univ Milan, Dipartimento Biotecnol & Biosci, I-20126 Milan, Italy
COUNTRY OF AUTHOR: Italy
SOURCE: BIOTECHNOLOGY LETTERS, (NOV 2001) Vol. 23, No. 21, pp. 1803-1808.
Publisher: KLUWER ACADEMIC PUBL, SPUIBOULEVARD 50, PO BOX 17, 3300 AA DORDRECHT, NETHERLANDS.
ISSN: 0141-5492.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 15
*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB Growth of C. **rugosa** on three different culture media was analysed by laser flow cytometry to evaluate physiological growth conditions allowing effective **lipase** production. The highest productivity was associated with an increased proportion of cells in the G1 phase and was independent of the effect of the medium on **lipase** formation.

L22 ANSWER 3 OF 59 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 2001:547452 SCISEARCH
THE GENUINE ARTICLE: 447RJ
TITLE: Crystal structure of 2-hydroxyl-6-oxo-6-phenylhexa-2,4-dienoic acid (HPDA) hydrolase (BphD enzyme) from the Rhodococcus sp strain RHA1 of the PCB degradation pathway
AUTHOR: Nandhagopal N; Yamada A; Hatta T; Masai E; Fukuda M; Mitsui Y; Senda T (Reprint)
CORPORATE SOURCE: Natl Inst Adv Ind Sci & Technol, Biol Informat Res Ctr, Kohto Ku, Tokyo 1350006, Japan (Reprint); Nagaoka Univ Technol, Dept Bioengn, Div Prot Engn, Niigata 9402188, Japan; Nagaoka Univ Technol, Dept Bioengn, Div Microbial Engn, Niigata 9402188, Japan; Okayama Univ, Res Inst Technol, Okayama 703, Japan
COUNTRY OF AUTHOR: Japan
SOURCE: JOURNAL OF MOLECULAR BIOLOGY, (22 JUN 2001) Vol. 309, No. 5, pp. 1139-1151.
Publisher: ACADEMIC PRESS LTD, 24-28 OVAL RD, LONDON NW1 7DX, ENGLAND.
ISSN: 0022-2836.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 50
*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB 2-Hydroxyl-6-oxo-6-phenylhexa-2, 2-dienoic acid (HPDA) hydrolase (the BphD enzyme) hydrolyzes a ring-cleavage product of an aromatic compound generated in a biphenyl/polychlorinated biphenyl (PCB) degradation pathway of bacteria. The crystal structure of the BphD enzyme has been determined at 2.4 Angstrom resolution by the multiple isomorphous replacement method. The final refined model of the BphD enzyme yields an R-factor of 17.5% at 2.4 Angstrom resolution with reasonable geometry. The BphD enzyme is an octameric enzyme with a 422 point-group symmetry. The subunit can be divided into core and lid domains. The active site of the enzyme is situated in the substrate-binding pocket, which is located between the two domains. The substrate-binding pocket can be divided into hydrophobic and hydrophilic regions. This feature of the pocket seems to be necessary for substrate binding, as the substrate is composed of hydrophilic and hydrophobic parts. The proposed orientation of the substrate seems to be consistent with the general catalytic mechanism of alpha/beta -hydrolases. (C) 2001 Academic Press.

L22 ANSWER 4 OF 59 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN

ACCESSION NUMBER: 2001:955751 SCISEARCH
THE GENUINE ARTICLE: 497MN
TITLE: Three new **lipases** from actinomycetes and their use in organic reactions
AUTHOR: Cardenas F; Alvarez E; De Castro-Alvarez M S; Sanchez-Montero J M; Elson S; Sinisterra J V (Reprint)
CORPORATE SOURCE: Univ Complutense Madrid, Fac Pharm, Dept Organ & Pharmaceut Chem, E-28040 Madrid, Spain (Reprint); SmithKline Beecham, Ctr Invest Basica, Madrid 28760, Spain
COUNTRY OF AUTHOR: Spain
SOURCE: BIOCATALYSIS AND BIOTRANSFORMATION, (29 NOV 2001) Vol. 19, No. 4, pp. 315-329.
Publisher: HARWOOD ACAD PUBL GMBH, TAYLOR & FRANCIS GROUP, 325 CHESTNUT ST, 8TH FL, PHILADELPHIA, PA 19106 USA.
ISSN: 1024-2422.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 25

*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB Three novel **lipase**-producing microorganisms have been isolated from 526 actinomycete strains by employing screening techniques on solid media, Time-course and scale-up of enzyme production were analyzed. The **lipases**, produced by microorganisms belonging to the Streptomyces genus, were tested in several reactions in organic medium using unnatural substrates. The lyophilized crude **lipases** are stable at least for 1 month at 4 degreesC (100% recovered activity). The **lipase** activity per milliliter of cell culture broth was higher than described in the literature for other **lipases** from actinomycetes. The three selected **lipases** displayed better activity than commercial **lipase** from Candida **rugosa** in the resolution of chiral secondary alcohols. The **lipase** from S. halstedii also displayed very good activity in the synthesis of carbamates.

L22 ANSWER 5 OF 59 MEDLINE on STN DUPLICATE 1

ACCESSION NUMBER: 2001686241 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11732718
TITLE: Production of native and recombinant **lipases** by Candida **rugosa**: a review.
AUTHOR: Ferrer P; Montesinos J L; Valero F; Sola C
CORPORATE SOURCE: Departament d'Enginyeria Quimica, ETSE, Universitat Autonoma de Barcelona, Bellaterra, Spain.
SOURCE: Applied biochemistry and biotechnology, (2001 Sep) 95 (3) 221-55. Ref: 152
Journal code: 8208561. ISSN: 0273-2289.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200206
ENTRY DATE: Entered STN: 20011205
Last Updated on STN: 20020620
Entered Medline: 20020619

AB The yeast Candida **rugosa** produces multiple **lipase** isoenzymes sharing high sequence homology but with some differences in their catalytic properties. The regulation of C. **rugosa** **lipase** (CRL) synthesis and secretion in C. **rugosa** obeys a complex pattern. Fermentation processes for both wild-type and mutant C. **rugosa** strains are available for **lipase** production. Native CRL preparations have been extensively used for biotransformations. However, their inherent mixture of isoforms with variable profiles complicates interpretation and brings into question the reproducibility achieved between preparations. Although heterologous CRLs **gene** expression had been hampered owing to a nonuniversal codon usage, recent advances have made heterologous CRLs available. This will expand and improve the industrial utility of CRLs even further. The purpose of this

review is to provide a summary of the recent advances on the production of native and recombinant **lipases** by C. **rugosa**.

L22 ANSWER 6 OF 59 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 2001269449 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11368188
TITLE: Recombinant expression and characterization of the Candida **rugosa** lip4 **lipase** in Pichia pastoris: comparison of glycosylation, activity, and stability.
AUTHOR: Tang S J; Shaw J F; Sun K H; Sun G H; Chang T Y; Lin C K; Lo Y C; Lee G C
CORPORATE SOURCE: Institute of Marine Biotechnology, National Taiwan Ocean University, Keelung, Republic of China.. tsj@mail.ntou.edu.tw
SOURCE: Archives of biochemistry and biophysics, (2001 Mar 1) 387 (1) 93-8.
Journal code: 0372430. ISSN: 0003-9861.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200106
ENTRY DATE: Entered STN: 20010618
Last Updated on STN: 20010618
Entered Medline: 20010614

AB Although Candida **rugosa** utilizes a nonuniversal serine codon (CUG) for leucine, it is possible to express **lipase genes** (LIP) in heterologous systems. After replacing the 19 CUG codons in LIP4 with serine codons by site-directed mutagenesis, a recombinant LIP4 was functionally overexpressed in Pichia pastoris in this study. This recombinant glycosylated **lipase** was secreted into the culture medium with a high purity of 100 mg/liter in a culture broth. Purified recombinant LIP4 had a molecular mass of 60 kDa, showing a range similar to that of **lipase** in a commercial preparation. Since LIP4 has only a glycosylation site at position Asn-351, this position may also be the major glycosylation site in C. **rugosa lipases**. Although the thermal stability of recombinant LIP4 significantly increased from 52 to 58 degrees C after glycosylation, there were no significant differences in the catalytic properties of recombinant glycosylated **lipase** from P. pastoris and the unglycosylated one from Escherichia coil. These two recombinant LIP4s showed higher esterase activities toward long-chain ester (C16 and C18) and exhibited higher **lipase** activities toward unsaturated and long-chain lipids. In addition, LIP4 does not show interfacial activation as compared with LIP1 toward lipid substrates of tributyrin and triolein. These observations demonstrated that LIP4 shows distinguished catalytic activities with LIP1 in spite of their high sequence homology.

L22 ANSWER 7 OF 59 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 2001:579628 SCISEARCH
THE GENUINE ARTICLE: 453ZJ
TITLE: Influence of the conformational flexibility on the kinetics and dimerisation process of two Candida **rugosa lipase** isoenzymes
AUTHOR: Pernas M A; Lopez C; Rua M L (Reprint); Hermoso J
CORPORATE SOURCE: Univ Vigo, Fac Ciencias Ourense, Area Bioquim & Biol Mol, As Lagoas S-N, Ourense 32004, Spain (Reprint); Univ Vigo, Fac Ciencias Ourense, Area Bioquim & Biol Mol, Ourense 32004, Spain; CSIC, Inst Rocasolano, Grp Cristalog Macromol & Biol Estructural, Madrid 28006, Spain
COUNTRY OF AUTHOR: Spain
SOURCE: FEBS LETTERS, (13 JUL 2001) Vol. 501, No. 1, pp. 87-91.
Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.
ISSN: 0014-5793.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 32

*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB We have investigated the interfacial activation process of two

isoenzymes from Candida **rugosa** (Lip1 and Lip3) using triacetin as substrate. Kinetics were coupled to inhibition experiments in order to analyse the transition between the open and closed conformers. This process was slow, particularly for Lip1, in the absence of an interface provided by the substrate or a detergent. Dimers of Lip3 were also purified and their catalytic action was closer to that of a typical esterase, In spite of the high sequence homology between Lip1 and Lip3, small changes enhance hydrophobicity in the binding pocket of Lip3 and increase the flexibility of its flap. We postulated that these factors account for the higher tendency of Lip3 to dimerise fixing its open conformation. (C) 2001 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.

L22 ANSWER 8 OF 59 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN

ACCESSION NUMBER: 2001:575995 SCISEARCH
THE GENUINE ARTICLE: 452RW
TITLE: Controlling **lipase** enantioselectivity for organic synthesis
AUTHOR: Berglund P (Reprint)
CORPORATE SOURCE: Royal Inst Technol, KTH, Dept Biotechnol, SE-10044 Stockholm, Sweden (Reprint)
COUNTRY OF AUTHOR: Sweden
SOURCE: BIOMOLECULAR ENGINEERING, (AUG 2001) Vol. 18, No. 1, pp. 13-22.
Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.
ISSN: 1389-0344.
DOCUMENT TYPE: General Review; Journal
LANGUAGE: English
REFERENCE COUNT: 136

*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB **Lipases** are used frequently as chiral catalysts in the synthesis of various fine chemicals and intermediates. The increasing need of compounds with high stereochemical purity requires catalysts with an improved and controlled performance. This overview emphasizes some important aspects for the control of **lipase** enantioselectivity and some examples where the enantioselectivity has been altered or reversed are highlighted. However, in several of these cases the complete explanation for the altered or reversed enantioselectivity remains unclear and needs to be solved. Three different strategies (engineering of the reaction medium, the substrate molecule, and the enzyme) for exploring **lipase** enantioselectivity at a molecular level are discussed and summarized. These three different approaches represent powerful tools for understanding the molecular basis for **lipase** enantioselective catalysis and can guide the rational improvement and tailoring of catalyst performance. By combining approaches from chemistry and biology much is learnt about the most important parameters controlling **lipase** enantioselectivity for organic synthesis. (C) 2001 Elsevier Science B.V. All rights reserved.

L22 ANSWER 9 OF 59 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN

ACCESSION NUMBER: 2001:264088 SCISEARCH
THE GENUINE ARTICLE: 413CA
TITLE: Effect of methanol and water contents on production of biodiesel fuel from plant oil catalyzed by various **lipases** in a solvent-free system
AUTHOR: Kaieda M; Samukawa T; Kondo A; Fukuda H (Reprint)
CORPORATE SOURCE: Kobe Univ, Fac Engn, Dept Sci & Chem Engn, Nada Ku, 1-1 Rokkodai Cho, Kobe, Hyogo 6578501, Japan (Reprint); Kobe Univ, Fac Engn, Dept Sci & Chem Engn, Nada Ku, Kobe, Hyogo 6578501, Japan; Kobe Univ, Grad Sch Sci & Technol, Div Mol Sci, Nada Ku, Kobe, Hyogo 6578501, Japan
COUNTRY OF AUTHOR: Japan
SOURCE: JOURNAL OF BIOSCIENCE AND BIOENGINEERING, (JAN 2001) Vol. 91, No. 1, pp. 12-15.
Publisher: SOC BIOSCIENCE BIOENGINEERING JAPAN, OSAKA UNIV, FACULTY ENGINEERING, 2-1 YAMADAOKA, SUITA, OSAKA, 565-0871, JAPAN.
ISSN: 1389-1723.

DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 7

*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB Methyl esters synthesized from plant oil and methanol by the methanolysis reaction are potentially important as a biodiesel fuel. The methanolysis of soybean oil by **lipases** from various microorganisms was investigated. Several of the **lipases** were found to catalyze methanolysis in a water-containing system without an organic solvent. The **lipases** from Candida **rugosa**, Pseudomonas cepacia, and Pseudomonas fluorescens displayed particularly high catalytic ability. The reaction rates of methanolysis catalyzed by the C. **rugosa** and P, fluorescens **lipases** decreased significantly when the water content was low, showing that water prevents the inactivation of these **lipases** by methanol, On the other hand, the methanolysis reaction rate catalyzed by the P, cepacia **lipase** remained high even under a low water content. In addition, the P, cepacia **lipase** gave high methyl ester contents in the reaction mixture up to 2 or 3 molar equivalents of methanol to oil, which is attributed to the P. cepacia **lipase** having substantial methanol resistance. For the same methanol content, the reaction rates of methanolysis catalyzed by the P. cepacia **lipase** increased with decreasing water content, and hence **lipases** strongly resistant to high methanol, such as that from P, cepacia, are desirable for use in methanolysis reaction processes.

L22 ANSWER 10 OF 59 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 2000:348037 SCISEARCH
THE GENUINE ARTICLE: 309JE
TITLE: Characterization of an extracellular **lipase** encoded by LIP2 in Yarrowia lipolytica
AUTHOR: Pignede G; Wang H J; Fudalej F; Gaillardin C; Seman M; Nicaud J M (Reprint)
CORPORATE SOURCE: INRA, LAB MICROBIOL & GENET MOL, GRIGNON CNRS, BP 01, F-78850 THIVERVAL GRIGNON, FRANCE (Reprint); INRA, LAB MICROBIOL & GENET MOL, GRIGNON CNRS, F-78850 THIVERVAL GRIGNON, FRANCE; LAB MAYOLY SPINDLER, SERV RECH, F-78401 CHATOU, FRANCE
COUNTRY OF AUTHOR: FRANCE
SOURCE: JOURNAL OF BACTERIOLOGY, (MAY 2000) Vol. 182, No. 10, pp. 2802-2810.
Publisher: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904.
ISSN: 0021-9193.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 52

*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB We isolated the LIP2 **gene** from the lipolytic yeast Yarrowia lipolytica. It was found to encode a 334-amino-acid precursor protein. The secreted **lipase** is a 301-amino-acid glycosylated polypeptide which is a member of the triacylglycerol hydrolase family (EC 3.1.1.3). The Lip2p precursor protein is processed by the KEX2-like endoprotease encoded by XPR6. Deletion of the XPR6 **gene** resulted in the secretion of an active but less stable proenzyme. Thus, the pro region does not inhibit **lipase** secretion and activity. However, it does play an essential role in the production of a stable enzyme. Processing was found to he correct in LIP2(A) (multiple LIP2 copy integrant)-overexpressing strains, which secreted 100 times more activity than the wild type, demonstrating that XPR6 maturation was not limiting. No extracellular **lipase** activity was detected with the lip2 knockout (KO) strain, strongly suggesting that extracellular **lipase** activity results from expression of the LIP2 **gene** . Nevertheless, the lip2 KO strain is still able to grow on triglycerides, suggesting an alternative pathway for triglyceride utilization in Y. lipolytica.

L22 ANSWER 11 OF 59 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN

ACCESSION NUMBER: 2000:414858 SCISEARCH
THE GENUINE ARTICLE: 317UM
TITLE: Mutants provide evidence of the importance of glycosydic chains in the activation of **lipase** 1 from Candida **rugosa**
AUTHOR: Brocca S; Persson M; Wehtje E; Adlercreutz P; Alberghina L; Lotti M (Reprint)
CORPORATE SOURCE: UNIV MILANO BICOCCA, DIPARTIMENTO BIOTECNOL & BIOSCI, PIAZZA SCI 2, I-20126 MILAN, ITALY (Reprint); UNIV MILANO BICOCCA, DIPARTIMENTO BIOTECNOL & BIOSCI, I-20126 MILAN, ITALY; LUND UNIV, DEPT BIOTECHNOL, S-22100 LUND, SWEDEN
COUNTRY OF AUTHOR: ITALY; SWEDEN
SOURCE: PROTEIN SCIENCE, (MAY 2000) Vol. 9, No. 5, pp. 985-990. Publisher: CAMBRIDGE UNIV PRESS, 40 WEST 20TH STREET, NEW YORK, NY 10011-4211. ISSN: 0961-8368.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 28

*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB Sequence analysis of Candida **rugosa lipase** 1 (LIP1) predicts the presence of three N-linked glycosylation sites at asparagine 291, 314, 351. To investigate the relevance of sugar chains in the activation and stabilization of LIP1, we directed site mutagenesis to replace the above mentioned asparagine with glutamine residues. Comparison of the activity of mutants with that of the wild-type (wt) **lipase** indicates that both 314 and 351 Asn to Gin substitutions influence, although at a different extent, the enzyme activity both in hydrolysis and esterification reactions, but they do not alter the enzyme water activity profiles in organic solvents or temperature stability. Introduction of Gin to replace Asn351 is likely to disrupt a stabilizing interaction between the sugar chain and residues of the inner side of the lid in the enzyme active conformation. The effect of deglycosylation at position 314 is more difficult to explain and might suggest a more general role of the sugar moiety for the structural stability of **lipase** 1. Conversely, Asn291Gln substitution does not affect the lipolytic or the esterase activity of the mutant that behaves essentially as the wt enzyme. This observation supports the hypothesis that changes in activity of Asn314Gln and Asn351Gln mutants are specifically due to deglycosylation.

L22 ANSWER 12 OF 59 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 20010732O6 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11049754
TITLE: Recombinant expression of the Candida **rugosa** lip4 **lipase** in Escherichia coli.
AUTHOR: Tang S J; Sun K H; Sun G H; Chang T Y; Lee G C
CORPORATE SOURCE: Institute of Marine Biotechnology, Keelung, 20224, Taiwan.. tsj@mail.ntou.edu.tw
SOURCE: Protein expression and purification, (2000 Nov) 20 (2) 308-13. Journal code: 9101496. ISSN: 1046-5928.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200101
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20010103

AB It is difficult to express recombinant Candida **rugosa lipases** (CRLs) in heterologous systems, since C. **rugosa** utilizes a nonuniversal serine codon CUG for leucine. In this study, recombinant LIP4 in which all 19 CUG codons had been converted to a universal serine codon was overexpressed in Escherichia coli BL21(DE3). The recombinant LIP4 was found mainly in the inclusion bodies and showed a low catalytic activity. To increase the amount of soluble form and activity of recombinant LIP4, the **DNA** was fused to the **gene** for thioredoxin (TrxFus-LIP4) and then expressed in E. coli strain AD494(DE3). This strategy promotes the formation of disulfide bonds in the cytosol and yields enzymatically active forms of LIP4. The

purified recombinant TrxFus-LIP4 and LIP4 expressed in AD494(DE3) had the same catalytic profiles. In addition, recombinant LIP4 had higher esterase activities toward long-chain ester and lower **lipase** activities toward tributyrin, triolein, and olive oil. This system for the expression of fungal **lipase** in E. coli strain AD494(DE3) is reliable and may produce enzymatically active forms of recombinant **lipase** without an in vitro refolding procedure.


L22 ANSWER 13 OF 59 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 2001:107432 BIOSIS
DOCUMENT NUMBER: PREV200100107432
TITLE: Purification and characterization of Lip2 and Lip3 isoenzymes from a Candida **rugosa** pilot-plant scale fed-batch fermentation.
AUTHOR(S): Pernas, M. A.; Lopez, C.; Pastrana, L.; Rua, M. L. [Reprint author]
CORPORATE SOURCE: Area de Bioquimica e Bioloxia Molecular, Facultade de Ciencias de Ourense, Universidade de Vigo, As Lagoas, 32004, Ourense, Spain
mlrua@uvigo.es
SOURCE: Journal of Biotechnology, (30 December, 2000) Vol. 84, No. 2, pp. 163-174. print.
CODEN: JBITD4. ISSN: 0168-1656.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 28 Feb 2001
Last Updated on STN: 15 Feb 2002
AB Previous purification of a crude extracellular enzyme preparation from Candida **rugosa** ATCC 14830 pilot-plant fed-batch fermentations showed the presence of two **lipase** isoenzymes, Lip2 and Lip3, differing in their molecular masses (58 and 62 kDa, respectively). These enzymes were purified but the **lipases** were forming active aggregates with a molecular mass higher than 200 kDa. In this work we developed a purification method following three steps: ammonium sulfate precipitation, sodium cholate treatment and ethanol/ether precipitation, and anion exchange chromatography which allowed the sequential disaggregation of the isoenzymes. Pure and monomeric Lip2 and Lip3 were characterized according to pI, glycosylation and activity for p-nitrophenol esters and triacylglycerols of varying acyl chain. Lip3 was the best catalyst for the hydrolysis of the simple esters and triacylglycerols with short and medium acyl chains.

L22 ANSWER 14 OF 59 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 2001:49257 BIOSIS
DOCUMENT NUMBER: PREV200100049257
TITLE: Design and realization of a tailor-made enzyme to modify the molecular recognition of 2-arylpropionic esters by Candida **rugosa lipase**.
AUTHOR(S): Manetti, Fabrizio; Mileto, Daniela; Corelli, Federico; Soro, Simonetta; Palocci, Cleofe; Cernia, Enrico; D'Acquarica, Ilaria; Lotti, Marina; Alberghina, Lilia; Botta, Maurizio [Reprint author]
CORPORATE SOURCE: Dipartimento Farmaco Chimico Tecnologico, Universita degli Studi di Siena, Via Aldo Moro snc, I-53100, Siena, Italy
botta@unisi.it
SOURCE: Biochimica et Biophysica Acta, (30 November, 2000) Vol. 1543, No. 1, pp. 146-158. print.
CODEN: BBACAQ. ISSN: 0006-3002.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 24 Jan 2001
Last Updated on STN: 12 Feb 2002
AB Within a research project aimed at probing the substrate specificity and the enantioselectivity of Candida **rugosa lipase** (CRL), computer modeling studies of the interactions between CRL and methyl (+-)-2-(3-benzoylphenyl)propionate (Ketoprofen methyl ester) have been carried out in order to identify which amino acids are essential to the enzyme/substrate interaction. Different binding models of the substrate

enantiomers to the active site of CRL were investigated by applying a computational protocol based on molecular docking, conformational analysis, and energy minimization procedures. The structural models of the computer generated complexes between CRL and the substrates enabled us to propose that Phe344 and Phe345, in addition to the residues constituting the catalytic triad and the oxyanion hole, are the amino acids mainly involved in the enzyme-ligand interactions. To test the importance of these residues for the enzymatic activity, site-directed mutagenesis of the selected amino acids has been performed, and the mutated enzymes have been evaluated for their conversion and selectivity capabilities toward different substrates. The experimental results obtained in these biotransformation reactions indicate that Phe344 and especially Phe345 influence CRL activity, supporting the findings of our theoretical simulations.

L22 ANSWER 15 OF 59 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2000:207906 CAPLUS
DOCUMENT NUMBER: 132:346651
TITLE: Reversible enzyme immobilization via a very strong and nondistorting ionic adsorption on support-polyethylenimine composites
AUTHOR(S): Mateo, Cesar; Abian, Olga; Fernandez-Lafuente, Roberto; Guisan, Jose M.
CORPORATE SOURCE: Departamento de Biocatalisis, Instituto de Catalisis, CSIC, Madrid, 28049, Spain
SOURCE: Biotechnology and Bioengineering (2000), 68(1), 98-105
CODEN: BIBIAU; ISSN: 0006-3592
PUBLISHER: John Wiley & Sons, Inc.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB New tailor-made anionic exchange resins have been prepd., based on films of large polyethylenimine polymers (e.g., mol. wt. 25,000) completely coating, via covalent immobilization, the surface of different porous supports (agarose, silica, polymeric resins). Most proteins contained in crude exts. from different sources have been very strongly adsorbed on them. Ionic exchange properties of such composites strongly depend on the size of polyethylenimine polymers as well as on the exact conditions of the covalent coating of the solids with the polymer. On the contrary, similar coating protocols yield similar matrixes by using different porous supports as starting material. For example, 77% of all proteins contained in crude exts. from Escherichia coli were adsorbed, at low ionic strength, on the best matrixes, and less than 15% of the adsorbed proteins were eluted from the support in the presence of 0.3 M NaCl. Under these conditions, 100% of the adsorbed proteins were eluted from conventional DEAE supports. Such polyethylenimine-support composites were also very suitable to perform very strong and nondistorting reversible immobilization of industrial enzymes. For example, **lipase** from Candida **rugosa** (CRL), .beta.-galactosidase from Aspergillus oryzae and D-amino acid oxidase (DAAO) from Rhodotorula gracilis, were adsorbed on such matrixes in a few minutes at pH 7.0 and 4.degree.C. Immobilized enzymes preserved 100% of catalytic activity and remained fully immobilized in 0.2 M NaCl. In addn. to that, CRL and DAAO were highly stabilized upon immobilization. Stabilization of DAAO, a dimeric enzyme, seems to be due to the involvement of both enzyme sub-units in the ionic adsorption.
REFERENCE COUNT: 25 THERE ARE 25 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L22 ANSWER 16 OF 59 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2000:222764 CAPLUS
DOCUMENT NUMBER: 133:55154
TITLE: Candida **rugosa lipases**: from molecular evolution analysis to the design of a synthetic **gene**
AUTHOR(S): Lotti, Marina; Alberghina, Lilia
CORPORATE SOURCE: Dipartimento Biotechnologie e Bioscienze, Universita Degli Studi Milano-Bicocca, Milan, 20126, Italy
SOURCE: Protein Engineering in Industrial Biotechnology (2000), 63-74. Editor(s): Alberghina, Lilia. Harwood Academic Publishers: Amsterdam, Neth.
CODEN: 68TUAF

DOCUMENT TYPE: Conference; General Review
LANGUAGE: English
AB A review with 60 refs. The family of **lipase** enzymes (CRLs) synthesized by the fungus Candida **rugosa** provides issues of interest for protein engineers because of a few unique features. First of all, C. **rugosa lipases** are members of a large set of proteins closely related in both sequence and function. In an evolutionary perspective, one can consider CRK proteins as the result of a successful process of mutagenesis where a defined group of amino acid changes have been pos. selected since they gave rise to functional variants. The study of **lipase** sequences therefore may provide a good starting point towards the design of functional mutants. On the other hand, C. **rugosa** belongs to a subgroup of Candida species which, using a non-conventional decoding of one serine codon, requires the development of novel approaches for the heterologous expression of its **genes**. Information arising may therefore be of interest for researchers using non-conventional expression systems.
REFERENCE COUNT: 60 THERE ARE 60 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L22 ANSWER 17 OF 59 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1999:220064 CAPLUS
DOCUMENT NUMBER: 130:247858
TITLE: A synthetic LIP1 **gene** of Candida **rugosa** with the codon usage of Pichia pastoris and the manufacture of the **lipase** encoded by the **gene**
INVENTOR(S): Brocca, Stefania; Schmidt-Dannert, Claudia; Lotti, Marina; Alberghina, Lilia; Schmid, Rolf
PATENT ASSIGNEE(S): Unilever N.V., Neth.
SOURCE: PCT Int. Appl., 44 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 9914338 | A1 | 19990325 | WO 1997-NL524 | 19970916 |
| W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | | |
| RW: GH, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG | | | | |
| AU 9742249 | A1 | 19990405 | AU 1997-42249 | 19970916 |
| EP 1012301 | A1 | 20000628 | EP 1997-940483 | 19970916 |
| R: BE, DE, DK, FR, GB, NL, SE | | | | |

PRIORITY APPLN. INFO.: WO 1997-NL524 A 19970916
AB The dimorphic yeast Candida **rugosa** has an unusual codon-usage which hampers the functional expression of **genes** derived from this yeast in a conventional heterologous host. **Lipases** produced by this yeast are extensively used in industrial bioconversions, but com. **lipase** samples contain several different isoforms encoded by the LIP **genes** family. In a first laborious attempt the LIP1 **gene**, encoding the major isoform of the C. **rugosa lipases** (CRLs), was systematically modified by site-directed mutagenesis to gain functional expression in S. cerevisiae. Mutagenesis was designed by homol. with other **lipases**. As alternative approach, the **gene** (1688 bp) was completely synthesized with an optimized nucleotide sequence in terms of heterologous expression in yeast and simplified genetic manipulation. The synthetic **gene** was overexpressed in Pichia pastoris. The **lipase** was produced at high level and purity, accounting for 90-95 % of the secreted proteins. The phys.-chem. and catalytic properties of the recombinant **lipase** were compared with those of a com. C. **rugosa lipase** prepn.
REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS

RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L22 ANSWER 18 OF 59 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 1999:938866 SCISEARCH
THE GENUINE ARTICLE: 260KL
TITLE: Switched enantiopreference of Humicola **lipase** for 2-phenoxyalkanoic acid ester homologs can be rationalized by different substrate binding modes
AUTHOR: Berglund P (Reprint); Vallikivi I; Fransson L; Dannacher H; Holmquist M; Martinelle M; Bjorkling F; Parve O; Hult K
CORPORATE SOURCE: ROYAL INST TECHNOL, DEPT BIOTECHNOL, SE-10044 STOCKHOLM, SWEDEN (Reprint); TALLINN TECH UNIV, INST CHEM, DEPT BIOORGAN CHEM, TALLINN 12618, ESTONIA; LEO PHARMACEUT PROD, DK-2750 BALLERUP, DENMARK
COUNTRY OF AUTHOR: SWEDEN; ESTONIA; DENMARK
SOURCE: TETRAHEDRON-ASYMMETRY, (29 OCT 1999) Vol. 10, No. 21, pp. 4191-4202.
Publisher: PERGAMON-ELSEVIER SCIENCE LTD, THE BOULEVARD, LANGFORD LANE, KIDLINGTON, OXFORD OX5 1GB, ENGLAND.
ISSN: 0957-4166.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: PHYS
LANGUAGE: English
REFERENCE COUNT: 32
*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB Humicola lanuginosa **lipase** was used for enantioselective hydrolyses of a series of homologous 2-phenoxyalkanoic acid ethyl esters. The enantioselectivity (E-value) of the enzyme changed from an (R)-enantiomer preference for the smallest substrate, 2-phenoxypropanoic acid ester, to an (S)-enantiomer preference for the homologous esters with longer acyl moieties. The E-values span the range from E=13 (R) to E=56 (S). A molecular modeling study identified two different substrate-binding modes for each enantiomer. We found that the enantiomers favored different modes. This discovery provided a model that offered a rational explanation for the observed switch in enantioselectivity. (C) 1999 Elsevier Science Ltd. All rights reserved.

L22 ANSWER 19 OF 59 MEDLINE on STN DUPLICATE 4
ACCESSION NUMBER: 1999402725 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10473391
TITLE: Analysis of the **gene** family encoding **lipases** in Candida **rugosa** by competitive reverse transcription-PCR.
AUTHOR: Lee G C; Tang S J; Sun K H; Shaw J F
CORPORATE SOURCE: Institute of Biochemistry, National Yang-Ming University, Taipei, Taiwan 11211.
SOURCE: Applied and environmental microbiology, (1999 Sep) 65 (9) 3888-95.
Journal code: 7605801. ISSN: 0099-2240.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF025307
ENTRY MONTH: 199912
ENTRY DATE: Entered STN: 20000113
Last Updated on STN: 20000113
Entered Medline: 19991223

AB Synthesis of multiple extracellular **lipases** in Candida **rugosa** has been demonstrated. However, it is difficult to characterize the expression spectrum of lip **genes**, since the sequences of the lip multigene family are very closely related. A competitive reverse transcription-PCR assay was developed to quantify the expression of lip **genes**. In agreement with the protein profile, the abundance of lip mRNAs was found to be (in decreasing order) lip1, lip3, lip2, lip5, and lip4. To analyze the effects of different culture conditions, the transcript concentrations for these mRNA species were normalized relative to the values for gpd, encoding glyceraldehyde-3-phosphate dehydrogenase. In relative terms, lip1 and lip3 were highly and constitutively expressed (about 10(5) molecules per microg of total RNA)

whereas the other inducible lip **genes**, especially lip4, showed significant changes in mRNA expression under different culture conditions. These results indicate that differential transcriptional control of lip **genes** results in multiple forms of **lipase** proteins.

L22 ANSWER 20 OF 59 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 1999:812285 SCISEARCH
THE GENUINE ARTICLE: 247EW
TITLE: Bacterial biocatalysts: Molecular biology, three-dimensional structures, and biotechnological applications of **lipases**
AUTHOR: Jaeger K E (Reprint); Dijkstra B W; Reetz M T
CORPORATE SOURCE: RUHR UNIV BOCHUM, LEHRSTUHL BIOL MIKROORGANISMEN, D-44780 BOCHUM, GERMANY (Reprint); UNIV GRONINGEN, BIOPHYS CHEM LAB, NL-9747 AG GRONINGEN, NETHERLANDS; MAX PLANCK INST KOHLENFORSCH, D-45470 MULHEIM, GERMANY
COUNTRY OF AUTHOR: GERMANY; NETHERLANDS
SOURCE: ANNUAL REVIEW OF MICROBIOLOGY, (JUL-AUG 1999) Vol. 53, pp. 315-&.
Publisher: ANNUAL REVIEWS INC, 4139 EL CAMINO WAY, PO BOX 10139, PALO ALTO, CA 94303-0139.
ISSN: 0066-4227.
DOCUMENT TYPE: General Review; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 169
*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB Bacteria produce and secrete **lipases**, which can catalyze both the hydrolysis and the synthesis of long-chain acylglycerols. These reactions usually proceed with high regioselectivity and enantioselectivity, and, therefore, **Lipases** have become very important stereoselective biocatalysts used in organic chemistry. High-level production of these biocatalysts requires the understanding of the mechanisms underlying **gene** expression, folding, and secretion. Transcription of **Lipase genes** may be regulated by quorum sensing and two-component systems; secretion can proceed either via the Sec-dependent general secretory pathway or via ABC transporters. In addition, some **lipases** need folding catalysts such as the **lipase**-specific foldases and disulfide-bond-forming proteins to achieve a secretion-competent conformation. Three-dimensional structures of bacterial **lipases** were solved to understand the catalytic mechanism of **lipase** reactions. Structural characteristics include an alpha/beta hydrolase fold, a catalytic triad consisting of a nucleophilic serine located in a highly conserved Gly-X-Ser-X-Gly pentapeptide, and an aspartate or glutamate residue that is hydrogen bonded to a histidine. Four substrate binding pockets were identified for triglycerides: an oxyanion hole and three pockets accommodating the fatty acids bound at positions sn-1, sn-2, and sn-3. The differences in size and the hydrophilicity/hydrophobicity of these pockets determine the enantiopreference of a **lipase**. The understanding of structure-function relationships will enable researchers to tailor new **lipases** for biotechnological applications. At the same time, directed evolution in combination with appropriate screening systems will be used extensively as a novel approach to develop **lipases** with high stability and enantioselectivity.

L22 ANSWER 21 OF 59 MEDLINE on STN DUPLICATE 5
ACCESSION NUMBER: 2000039909 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10570248
TITLE: Structure and conformational flexibility of Candida **rugosa lipase**.
AUTHOR: Cygler M; Schrag J D
CORPORATE SOURCE: Biotechnology Research Institute, National Research Council of Canada, 6100 Royalmount Avenue, Montreal, Quebec, Canada.. mirek.cygler@bri.nrc.ca
SOURCE: Biochimica et biophysica acta, (1999 Nov 23) 1441 (2-3) 205-14. Ref: 23
Journal code: 0217513. ISSN: 0006-3002.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199912
ENTRY DATE: Entered STN: 20000114
Last Updated on STN: 20000114
Entered Medline: 19991230

AB Three-dimensional structures of a number of **lipases** determined in the past decade have provided a solid structural foundation for our understanding of **lipase** function. The structural studies of Candida **rugosa lipase** summarized here have addressed many facets of interfacial catalysis. These studies have revealed a fold and catalytic site common to other **lipases**. Different conformations likely to correlate with interfacial activation of the enzyme were observed in different crystal forms. The structures of enzyme-inhibitor complexes have identified the binding site for the scissile fatty acyl chain, provided the basis for molecular modeling of triglyceride binding and provided insight into the structural basis of the common enantiopreferences shown by **lipases**.

L22 ANSWER 22 OF 59 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 1999:413237 SCISEARCH
THE GENUINE ARTICLE: 198XT
TITLE: A controlled fed-batch cultivation for the production of new crude **lipases** from Candida **rugosa** with improved properties in fine chemistry
AUTHOR: Sanchez A; Ferrer P; Serrano A; Valero F (Reprint); Sola C; Pernas M; Rua M L; FernandezLafuente R; Guisan J M; delaCasa R; Sinisterra J V; SanchezMontero J M
CORPORATE SOURCE: UNIV AUTONOMA BARCELONA, DETP ENGN QUIM, E-08193 BARCELONA, SPAIN (Reprint); UNIV AUTONOMA BARCELONA, DETP ENGN QUIM, E-08193 BARCELONA, SPAIN; UNIV VIGO, FAC CIENCIAS OURENSE, AREA BIOQUIM & BIOLOXIA MOL, ORENSE 32004, SPAIN; UNIV COMPLUTENSE, FAC FARM, DEPT QUIM ORGAN & FARMACEUT, E-28040 MADRID, SPAIN; CSIC, INST CATALISIS, LAB TECNOL ENZIMAT, E-28049 MADRID, SPAIN
COUNTRY OF AUTHOR: SPAIN
SOURCE: JOURNAL OF BIOTECHNOLOGY, (15 APR 1999) Vol. 69, No. 2-3, pp. 169-182.
Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.
ISSN: 0168-1656.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE; AGRI
LANGUAGE: English
REFERENCE COUNT: 43
*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB A controlled constant feeding rate fed-batch strategy using oleic acid as inducer produced a crude **lipase** preparation from Candida **rugosa** (CRL-UAB) with higher protein content, carbohydrate content and **lipase** activity than commercial Sigma type VII CRL. CRL-UAB was partially characterised and tested in selective biotransformations of chiral compounds in aqueous (2-hydroxy 4-phenyl butanoic acid ethyl ester (HPBE)) and organic media (2-phenyl propionic acid and ketoprofen). CRL-UAB showed higher substrate specificity and enantioselectivity in aqueous media compared to Sigma CRL. Also, higher specific initial rates with 2-phenyl propionic acid and ketoprofen were observed in organic media. The influence of water on the esterification of ketoprofen was not relevant with CRL-UAB under the conditions tested, whereas a dramatic influence was observed in Sigma CRL. Different CRL-UAB batches obtained under the same cultivation controlled conditions were identical from the point of view of chromatographic behaviour, immobilisation rates and catalytic properties, indicating that a reproducible C. **rugosa** **lipase** extract had been obtained. 

L22 ANSWER 23 OF 59 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 1998:915281 SCISEARCH

THE GENUINE ARTICLE: 142NR
TITLE: Structure and activity of rat pancreatic **lipase** -related protein 2
AUTHOR: Roussel A; Yang Y Q; Ferrato F; Verger R; Cambillau C; Lowe M (Reprint)
CORPORATE SOURCE: WASHINGTON UNIV, SCH MED, DEPT PEDIAT, ST LOUIS, MO 63110 (Reprint); WASHINGTON UNIV, SCH MED, DEPT PEDIAT, ST LOUIS, MO 63110; WASHINGTON UNIV, SCH MED, DEPT MOL BIOL & PHARMACOL, ST LOUIS, MO 63110; CNRS, IFR1, UPR 9039, F-13402 MARSEILLE 20, FRANCE; CNRS, IFR1, UPR 9025, LAB LIPOLYSE ENZYMAT, F-13402 MARSEILLE 20, FRANCE
COUNTRY OF AUTHOR: USA; FRANCE
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (27 NOV 1998) Vol. 273, No. 48, pp. 32121-32128.
Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814.
ISSN: 0021-9258.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 39
*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB The pancreas expresses several members of the **lipase** **gene** family including pancreatic triglyceride **lipase** (PTL) and two homologous proteins, pancreatic **lipase**-related proteins 1 and 2 (PLRP1 and PLRP2), Despite their similar amino acid sequences, PTL, PLRP1, and PLRP2 differ in important kinetic properties. PLRP1 has no known activity. PTL and PLRP2 differ in substrate specificity, bile acid inhibition, colipase requirement, and interfacial activation. To begin understanding the structural explanations for these functional differences, we solved the crystal structure of rat (r)PLRP2 and further characterized its kinetic properties. The 1.8 Angstrom structure of rPLRP2, like the tertiary structure of human PTL, has a globular N-terminal domain and a beta-sandwich C-terminal domain. The lid domain occupied the closed position, suggesting that rPLRP2 should show interfacial activation. When we reexamined this issue with tripropionin as substrate, rPLRP2 exhibited interfacial activation. Because the active site topology of rPLRP2 resembled that of human PTL, we predicted and demonstrated that the **lipase** inhibitors E600 and tetrahydrolipstatin inhibit rPLRP2, Although PTL and rPLRP2 have similar active sites, rPLRP2 has a broader substrate specificity that we confirmed using a monolayer technique, With this assay, we showed for the first time that rPLRP2 prefers phosphatidylglycerol and ethanolamine over phosphatidylcholine. In summary, we confirmed and extended the observation that PLRP2 **lipases** have a broader substrate specificity than PTL, we demonstrated that PLRP2 **lipases** show interfacial activation, and we solved the first crystal structure of a PLRP2 **lipase** that contains a lid domain.

L22 ANSWER 24 OF 59 MEDLINE on STN DUPLICATE 6
ACCESSION NUMBER: 1998318052 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9655346
TITLE: Design, total synthesis, and functional overexpression of the Candida **rugosa** **lip1** **gene** coding for a major industrial **lipase**.
AUTHOR: Brocca S; Schmidt-Dannert C; Lotti M; Alberghina L; Schmid R D
CORPORATE SOURCE: Institut fur Technische Biochemie, Universitat Stuttgart, Germany.
SOURCE: Protein science : a publication of the Protein Society, (1998 Jun) 7 (6) 1415-22.
Journal code: 9211750. ISSN: 0961-8368.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199809
ENTRY DATE: Entered STN: 19980910
Last Updated on STN: 19980910
Entered Medline: 19980901

AB The dimorphic yeast Candida **rugosa** has an unusual codon usage

that hampers the functional expression of **genes** derived from this yeast in a conventional heterologous host. Commercial samples of C. **rugosa lipase** (CRL) are widely used in industry, but contain several different isoforms encoded by the lip **gene** family, among which the isoform encoded by the **gene** lip1 is the most prominent. In a first laborious attempt, the lip1 **gene** was systematically modified by site-directed mutagenesis to gain functional expression in Saccharomyces cerevisiae. As alternative approach, the **gene** (1647 bp) was completely synthesized with an optimized nucleotide sequence in terms of heterologous expression in yeast and simplified genetic manipulation. The synthetic **gene** was functionally expressed in both hosts S. cerevisiae and Pichia pastoris, and the effect of heterologous leader sequences on expression and secretion was investigated. In particular, using P. pastoris cells, the synthetic **gene** was functionally overexpressed, allowing for the first time to produce recombinant Lip1 of high purity at a level of 150 U/mL culture medium. The physicochemical and catalytic properties of the recombinant **lipase** were compared with those of a commercial, nonrecombinant C. **rugosa lipase** preparation containing **lipase** isoforms.

L22 ANSWER 25 OF 59 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN

ACCESSION NUMBER: 1998:132513 SCISEARCH
THE GENUINE ARTICLE: YV746
TITLE: A cold-adapted **lipase** of an Alaskan psychrotroph, Pseudomonas sp. strain B11-1: **Gene** cloning and enzyme purification and characterization
AUTHOR: Choo D W; Kurihara T; Suzuki T; Soda K; Esaki N (Reprint)
CORPORATE SOURCE: KYOTO UNIV, INST CHEM RES, KYOTO 611, JAPAN (Reprint); KYOTO UNIV, INST CHEM RES, KYOTO 611, JAPAN; KANSAI UNIV, FAC ENGN, OSAKA 564, JAPAN
COUNTRY OF AUTHOR: JAPAN
SOURCE: APPLIED AND ENVIRONMENTAL MICROBIOLOGY, (FEB 1998) Vol. 64, No. 2, pp. 486-491.
Publisher: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171.
ISSN: 0099-2240.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE; AGRI
LANGUAGE: English
REFERENCE COUNT: 44

*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB A psychrotrophic bacterium producing a cold-adapted **lipase** upon growth th at low temperatures was isolated from Alaskan soil and identified as a Pseudomonas strain, The **lipase gene** (lipP) was cloned from the strain and sequenced, The amino acid sequence deduced from the nucleotide sequence of the **gene** (924 bp) corresponded to a protein of 308 amino acid residues with a molecular weight of 33,714. LipP also has consensus motifs conserved in other cold-adapted **lipases**, i.e., **Lipase** 2 from Antarctic Moraxella TA144 (G. Feller, M. Thiry, J. L. Arpigny, and C. Gerday, **DNA** Cell Biol. 10:381-388, 1991) and the mammalian hormone-sensitive **lipase** (D. Langin, H. Laurell, L. S. Hoist, P. Belfrage, and C. Holm, Proc. Natl. Acad. Sci. USA 90:4897-4901, 1993): a pentapeptide, GDSAG, containing the putative active-site serine and an BG dipeptide, LipP was purified from an extract of recombinant Escherichia coli C600 cells harboring a plasmid coding for the lipP **gene**, The enzyme showed a 1,3-positional specificity toward triolein, p-Nitrophenyl esters of fatty acids with short to medium chains (C-4 and C-6) served as good substrates. The enzyme was stable between pH 6 and 9, and the optimal pH for the enzymatic hydrolysis of tributyrin was around 8, The activation energies for the hydrolysis of p-nitrophenyl butyrate and p-nitrophenyl laurate were determined to be 11.2 and 7.7 kcal/mol, respectively. in the temperature range 5 to 35 degrees C, The enzyme was unstable at temperatures higher than 45 degrees C. The K-m of the enzyme for p-nitrophenyl butyrate increased with increases in the assay temperature, The enzyme was strongly inhibited by Zn2+, Cu2+, Fe3+, and Hg2+ but was not affected by phenylmethylsulfonyl fluoride and bis-nitrophenyl phosphate, Various water-miscible organic solvents, such as methanol and dimethyl sulfoxide, at concentrations of 0 to 30%

(vol/vol) activated the enzyme.

L22 ANSWER 26 OF 59 MEDLINE on STN DUPLICATE 7
ACCESSION NUMBER: 1998386725 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9720256
TITLE: Physiological control on the expression and secretion of Candida **rugosa lipase**.
AUTHOR: Lotti M; Monticelli S; Montesinos J L; Brocca S; Valero F; Lafuente J
CORPORATE SOURCE: Dipartimento di Fisiologia e Biochimica Generali, Universita degli Studi di Milano, Italy.. lotti@imiucca.csi.unimi.it
SOURCE: Chemistry and physics of lipids, (1998 Jun) 93 (1-2) 143-8. Journal code: 0067206. ISSN: 0009-3084.
PUB. COUNTRY: Ireland
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199809
ENTRY DATE: Entered STN: 19981006
Last Updated on STN: 19981006
Entered Medline: 19980918

AB The fungus Candida **rugosa** secretes an extracellular **lipase** whose production is induced by the addition of fatty acids to the culture broth. This **lipase** is indeed composed by several protein isoforms partly differing in their catalytic properties. Synthesis and secretion of **lipase** proteins by C. **rugosa** cells were studied in culture media containing either glucose or oleic acid as the carbon source. It was shown that, according to their regulation, **lipase**-encoding **genes** might be grouped in two classes, one of which is constitutively expressed and the other is induced by fatty acids. The synthesis of inducible enzymes is inhibited at the level of transcription by the addition of glucose and, conversely, oleic acid appears to hinder the synthesis of the constitutive **lipase**. Growth conditions supporting high level expression both in batch and in continuous culture give rise to the intracellular accumulation of enzyme, possibly due to the existence of a rate-limiting step in the transport of the newly synthesized protein. These results suggest the possibility to develop fermentation processes aimed at the control of the enzyme composition.

L22 ANSWER 27 OF 59 MEDLINE on STN DUPLICATE 8
ACCESSION NUMBER: 1998386718 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9720249
TITLE: Characterization of the Candida **rugosa** **lipase** system and overexpression of the lip1 isoenzyme in a non-conventional yeast.
AUTHOR: Mileto D; Brocca S; Lotti M; Takagi M; Alquati C; Alberghina L
CORPORATE SOURCE: Department of General Physiology and Biochemistry, University of Milano, Italy.
SOURCE: Chemistry and physics of lipids, (1998 Jun) 93 (1-2) 47-55. Ref: 40
Journal code: 0067206. ISSN: 0009-3084.
PUB. COUNTRY: Ireland
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199809
ENTRY DATE: Entered STN: 19981006
Last Updated on STN: 19981006
Entered Medline: 19980918

AB The fungus C. **rugosa** produces **lipase** isoenzymes (CRLs) homologous to the Geotrichum candidum and Yarrowia lipolytica **lipases** to which they share ca. 40 and 30% sequence identity, with a domain of sequence conservation at the N-terminal half of the protein. CRL proteins have high sequence homology but are not identical in their catalytic activity, therefore calling for the resolution of isoforms via heterologous expression. The non-conventional use of a serine codon in

several Candida species frustrates overexpression in the currently available host systems. The LIP1 **gene**, coding for the major CRL form, was therefore expressed in C. maltosa, a related fungus with the same codon usage as C. **rugosa**. A recombinant **lipase** was produced and secreted in an active form in the culture medium upon engineering the 5' and 3' ends of the **gene**.

L22 ANSWER 28 OF 59 MEDLINE on STN DUPLICATE 9
ACCESSION NUMBER: 1999127041 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9928080
TITLE: A toolbox of recombinant **lipases** for industrial applications.
AUTHOR: Schmidt-Dannert C; Pleiss J; Schmid R D
CORPORATE SOURCE: Institute of Technical Biochemistry, University of Stuttgart, Germany.
SOURCE: Annals of the New York Academy of Sciences, (1998 Dec 13) 864 14-22. Ref: 27
Journal code: 7506858. ISSN: 0077-8923.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199903
ENTRY DATE: Entered STN: 19990324
Last Updated on STN: 19990324
Entered Medline: 19990309

AB We created a toolbox of recombinant, microbial **lipases**, which allows us in combination with a **lipase** database to choose among the overexpressed **lipases** the most appropriate for a specific application and to improve it further via mutagenesis. By systematic comparison of geometry and properties of the scissile fatty acid binding site of five representative **lipases** of each family of structurally homologous **lipases**, three subgroups can be defined. Hence, efficient expression systems for the functional production of large amounts of microbial **lipases**, representing different **lipase** subgroups, were developed. In particular, recombinant **lipases** from Bacillus thermocatenulatus and Pseudomonas cepacia were functionally overexpressed in E. coli. The **lipase genes** from Geotrichum candidum CMICC 335426 and Rhizopus oryzae were overexpressed in Pichia pastoris. Due to an unusual codon usage that prevents heterologous expression, the LIP1 **gene** (1647 nt) of Candida **rugosa** was completely synthesized and overexpressed in Pichia pastoris.

L22 ANSWER 29 OF 59 MEDLINE on STN DUPLICATE 10
ACCESSION NUMBER: 1998112187 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9450388
TITLE: Molecular cloning of **cDNA** for the zeta isoform of the 14-3-3 protein: homologous sequences in the 3'-untranslated region of frog and human zeta isoforms.
AUTHOR: Miura I; Nakajima T; Ohtani H; Kashiwagi A; Nakamura M
CORPORATE SOURCE: Laboratory for Amphibian Biology, Faculty of Science, Hiroshima University, Japan.
SOURCE: Zoological science, (1997 Oct) 14 (5) 771-5.
Journal code: 8702287. ISSN: 0289-0003.
PUB. COUNTRY: Japan
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Biotechnology
ENTRY MONTH: 199802
ENTRY DATE: Entered STN: 19980306
Last Updated on STN: 19980306
Entered Medline: 19980224

AB 14-3-3 proteins constitute a family of well-conserved eukaryotic proteins that possess diverse biochemical activities such as regulation of **gene** transcription, cell proliferation and activation of protein kinase C. At least 7 subtypes (alpha to theta) of 14-3-3 protein are known, but the zeta subtype of this protein has been cloned only in mammals. We cloned the zeta subtype of 14-3-3 protein (14-3-3 zeta) from

the frog, Rana **rugosa**. The sequence encoded 245 amino acids that share 92% identity with rat and bovine 14-3-3 zeta s, and 92% with human **phospholipase** A2 (PLA2; 14-3-3 zeta). Northern blot analysis revealed a single band of about 1.8 kb in tadpoles at stage 25. The 14-3-3 zeta mRNA level was high in the brain, lung, spleen and kidney, and low in the heart and testis, as opposed to the mRNA level, which was only faintly detected in the liver, pancreas, ovary and muscle. Furthermore, high similarity in the 3'-untranslated region (3'-UTR) was observed between frog and human 14-3-3 zeta **cDNA**. The results suggest that 14-3-3 zeta is highly conserved throughout eukaryotic evolution, and that the homologous sequence in the 3'-UTR of 14-3-3 zeta **cDNA** may be conserved in frogs and humans.

L22 ANSWER 30 OF 59 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 97:769505 SCISEARCH
THE GENUINE ARTICLE: BJ65Z
TITLE: Kinetics, molecular modeling, and synthetic applications with microbial **lipases**
AUTHOR: Hult K (Reprint); Holmquist M
CORPORATE SOURCE: ROYAL INST TECHNOL, DEPT BIOCHEM & BIOTECHNOL, S-10044 STOCKHOLM, SWEDEN (Reprint)
COUNTRY OF AUTHOR: SWEDEN
SOURCE: METHODS IN ENZYMOLOGY, (30 SEP 1997) Vol. 286, pp. 386-405

Publisher: ACADEMIC PRESS INC, 525 B STREET, SUITE 1900, SAN DIEGO, CA 92101-4495.
ISSN: 0076-6879.
DOCUMENT TYPE: General Review; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 47

L22 ANSWER 31 OF 59 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 97:728198 SCISEARCH
THE GENUINE ARTICLE: BJ57Q
TITLE: Stereoselectivity of **lipase** from Rhizopus oryzae toward triacylglycerols and analogs: Computer-aided modeling and experimental validation
AUTHOR: Haalck L (Reprint); Paltauf F; Pleiss J; Schmid R D; Spener F; Stadler P
CORPORATE SOURCE: INST CHEM & BIOCHEM SENSOR RES, D-48149 MUNSTER, GERMANY (Reprint); GRAZ TECH UNIV, DEPT BIOCHEM & FOOD CHEM, A-8010 GRAZ, AUSTRIA; UNIV STUTTGART, INST TECH BIOCHEM, D-70569 STUTTGART, GERMANY
COUNTRY OF AUTHOR: GERMANY; AUSTRIA
SOURCE: METHODS IN ENZYMOLOGY, (15 SEP 1997) Vol. 284, pp. 353-376

Publisher: ACADEMIC PRESS INC, 525 B STREET, SUITE 1900, SAN DIEGO, CA 92101-4495.
ISSN: 0076-6879.
DOCUMENT TYPE: General Review; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 40

L22 ANSWER 32 OF 59 CAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 11
ACCESSION NUMBER: 1997:712688 CAPLUS
DOCUMENT NUMBER: 128:11432
TITLE: Cloning, sequencing, and expression of Candida **rugosa lipases**
AUTHOR(S): Alberghina, Lilia; Lotti, Marina
CORPORATE SOURCE: USA
SOURCE: Methods in Enzymology (1997), 284(Lipases, Part A), 246-260
CODEN: MENZAU; ISSN: 0076-6879
PUBLISHER: Academic
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The authors describe work concerning the cloning and expression of Candida

**rugosa lipases**, which are peculiar among fungal **lipase** for two reasons: (1)the large no. of encoding **genes** and (2) a deviation from the universal genetic code in the source yeast. The importance and effects of this phenomenon for **lipase** structure and function, as well as for the expression of cloned **genes** in host systems, are discussed.

REFERENCE COUNT: 25 THERE ARE 25 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L22 ANSWER 33 OF 59 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN

ACCESSION NUMBER: 97:186489 SCISEARCH
THE GENUINE ARTICLE: WK598
TITLE: The crystal structure of a triacylglycerol **lipase** from Pseudomonas cepacia reveals a highly open conformation in the absence of a bound inhibitor
AUTHOR: Kim K K; Song H K; Shin D H; Hwang K Y; Suh S W (Reprint)
CORPORATE SOURCE: SEOUL NATL UNIV, COLL NAT SCI, DEPT CHEM, SEOUL 151742, SOUTH KOREA (Reprint); SEOUL NATL UNIV, COLL NAT SCI, DEPT CHEM, SEOUL 151742, SOUTH KOREA
COUNTRY OF AUTHOR: SOUTH KOREA
SOURCE: STRUCTURE, (15 FEB 1997) Vol. 5, No. 2, pp. 173-185. Publisher: CURRENT BIOLOGY LTD, 34-42 CLEVELAND STREET, LONDON, ENGLAND W1P 6LB. ISSN: 0969-2126.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 61

*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB Background: **Lipases**, a family of enzymes which catalyze the hydrolysis of triglycerides, are widely distributed in many organisms. True **lipases** are distinguished from esterases by the characteristic interfacial activation they exhibit at an oil-water interface. **Lipases** are one of the most frequently used biocatalysts for organic reactions performed under mild conditions, Their biotechnological applications include food and oil processing and the preparation of chiral intermediates for the synthesis of enantiomerically pure pharmaceuticals. Recent structural studies on several **lipases** have provided some clues towards understanding the mechanisms of hydrolytic activity, interfacial activation, and stereoselectivity. This study was undertaken in order to provide structural information on bacterial **lipases**, which is relatively limited in comparison to that on the enzymes from other sources.

Results: We have determined the crystal structure of a triacylglycerol **lipase** from Pseudomonas cepacia (Pet) in the absence of a bound inhibitor using X-ray crystallography, The structure shows the **lipase** to contain an alpha/beta-hydrolase fold and a catalytic triad comprising of residues Ser87, His286 and Asp264. The enzyme shares several structural features with homologous **lipases** from Pseudomonas glumae (PgL) and Chromobacterium viscosum (CvL), including a calcium-binding site, The present structure of Pet reveals a highly open conformation with a solvent-accessible active site, This is in contrast to the structures of PgL and Pet in which the active site is buried under a closed or partially opened 'lid', respectively.

Conclusions: Pet exhibits some structural features found in other **lipases**. The presence of the Ser-His-Asp catalytic triad, an oxyanion hole, and the opening of a helical lid suggest that this enzyme shares the same mechanisms of catalysis and interfacial activation as other **lipases**. The highly open conformation observed in this study is likely to reflect the activated form of the **lipase** at an oil-water interface. The structure suggests that the interfacial activation of bacterial **lipases** involves the reorganization of secondary structures and a large movement of the lid to expose the active site, This is similar to the mechanism described for other well characterized fungal and mammalian **lipases**.

L22 ANSWER 34 OF 59 CAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 12

ACCESSION NUMBER: 1997:343890 CAPLUS
DOCUMENT NUMBER: 127:61390
TITLE: The evolution of a non universal codon as detected in

Candida **rugosa lipase**
AUTHOR(S): Alberghina, Lilia; Lotti, Marina
CORPORATE SOURCE: Department of General Physiology and Biochemistry, University of Milano, Milan, I-20133, Italy
SOURCE: Journal of Molecular Catalysis B: Enzymatic (1997), 3(1-4), 37-41
CODEN: JMCEF8; ISSN: 1381-1177
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English

AB In several Candida species belonging to the same monophyletic group, CUG - one of the six triplets coding leucine in the universal genetic code - is read as an addnl. codon for serine. CUG for serine is rarely employed but in the **lipase genes** of Candida **rugosa**, where it is the major serine codon. This yeast secretes multiple **lipase** isoenzymes (CRLs) tightly related in their amino acid sequence. CRL proteins contain 16 to 19 CUG-serines comprehensive of Ser 209 in the catalytic center and other serines having an obvious structural role. In this paper, results obtained from sequence anal. and mutagenesis are discussed and shown to be consistent with an evolutionary pathway in which the codon was reassigned via ambiguous decoding by a novel seryl-tRNA whose abundance conferred pos. selection pressure for the extensive use of CUG as a serine codon.

REFERENCE COUNT: 16 THERE ARE 16 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L22 ANSWER 35 OF 59 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 97:740032 SCISEARCH
THE GENUINE ARTICLE: XY802
TITLE: High-level production of recombinant Geotrichum candidum **lipases** in yeast Pichia pastoris
AUTHOR: Holmquist M (Reprint); Tessier D C; Cygler M
CORPORATE SOURCE: NATL RES COUNCIL CANADA, BIOTECHNOL RES INST, 6100 ROYALMOUNT AVE, MONTREAL, PQ H4P 2R2, CANADA (Reprint); ROYAL INST TECHNOL, DEPT BIOCHEM & BIOTECHNOL, S-10044 STOCKHOLM, SWEDEN
COUNTRY OF AUTHOR: CANADA; SWEDEN
SOURCE: PROTEIN EXPRESSION AND PURIFICATION, (OCT 1997) Vol. 11, No. 1, pp. 35-40.
Publisher: ACADEMIC PRESS INC JNL-COMP SUBSCRIPTIONS, 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495.
ISSN: 1046-5928.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 30

*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB We describe the heterologous high-level expression of the two Geotrichum candidum **lipase** (GCL) isoenzymes from strain ATCC 34614 in the methylotrophic yeast Pichia pastoris. The **Lipase cDNAs** were placed under the control of the methanol-inducible alcohol oxidase promoter. The **lipases** expressed in P. pastoris were fused to the a-factor secretion signal peptide of Saccharomyces cerevisiae and were secreted into the culture medium. Cultures of P. pastoris expressing **lipase** accumulated active recombinant enzyme in the supernatant to levels of similar to 60 mg/L virtually free from contaminating proteins. This yield exceeds that previously reported with S. cerevisiae by a factor of more than 60. Recombinant GCL I and GCL II had molecular masses of similar to 63 and similar to 66 kDa, respectively, as determined by SDS-PAGE. The result of endoglucosidase H digestion followed by Western blot analysis of the **lipases** suggested that the enzymes expressed in P. pastoris received N-linked high-mannose-type glycosylation to an extent, 6-8% (w/w), similar to that in G. candidum. The specific activities and substrate specificities of both recombinant **lipases** were determined and were found to agree with what has been reported for the enzymes isolated from the native source. (C) 1997 Academic Press.

L22 ANSWER 36 OF 59 MEDLINE on STN DUPLICATE 13
ACCESSION NUMBER: 1998051607 MEDLINE

DOCUMENT NUMBER: PubMed ID: 9390168
TITLE: Isolation of carboxylester **lipase** (CEL) isoenzymes from Candida **rugosa** and identification of the corresponding **genes**.
AUTHOR: Diczfalusy M A; Hellman U; Alexson S E
CORPORATE SOURCE: Department of Medical Laboratory Sciences and Technology, Karolinska Institutet, Huddinge University Hospital, Sweden.
SOURCE: Archives of biochemistry and biophysics, (1997 Dec 1) 348 (1) 1-8.
Journal code: 0372430. ISSN: 0003-9861.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199712
ENTRY DATE: Entered STN: 19980116
Last Updated on STN: 19980116
Entered Medline: 19971224

AB The yeast Candida **rugosa** produces extracellular **lipases** which are widely used for industrial purposes. A commercial **lipase** preparation from this yeast can be separated into several isoenzymes which differ in carbohydrate content, isolelectric point, substrate specificity, and primary sequence. We have here purified and characterized three **lipases**, which also hydrolyze p-nitrophenyl esters, from a commercial preparation of this yeast. These three carboxylester **lipases** (CELs) elute differently on hydrophobic interaction chromatography, and have different carbohydrate contents and substrate specificities. Sequence analysis of their amino termini and peptides generated by LysC treatment showed that CEL-1 and CEL-3 probably have identical primary structure while CEL-2 was proven to be a different enzyme. Sequence comparison showed that both CEL-1 and CEL-3 are products of the LIP1 **gene** and that CEL-2 is the **gene** product of LIP2, cloned by Longhi et al. (Biochim. Biophys. Acta 1131, 227-232, 1992).

L22 ANSWER 37 OF 59 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1996:304153 CAPLUS
DOCUMENT NUMBER: 124:334869
TITLE: Transgenic oil plants expressing a foreign **lipase gene** and their use in the manufacture of free fatty acids
INVENTOR(S): Alibert, Gilbert; Mouloungui, Zephirin; Boudet, Alain
PATENT ASSIGNEE(S): Institut National Polytechnique de Toulouse (I.N.P.T.), Fr.
SOURCE: PCT Int. Appl., 31 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: French
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 9603511 | A2 | 19960208 | WO 1995-FR957 | 19950718 |
| WO 9603511 | A3 | 19960425 | | |
| W: AU, BG, CA, CN, HU, JP, NZ, PL, RO, RU, US | | | | |
| RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE | | | | |
| FR 2722798 | A1 | 19960126 | FR 1994-9272 | 19940725 |
| FR 2722798 | B1 | 19960913 | | |
| CA 2195560 | AA | 19960208 | CA 1995-2195560 | 19950718 |
| AU 9529849 | A1 | 19960222 | AU 1995-29849 | 19950718 |
| EP 770134 | A2 | 19970502 | EP 1995-925897 | 19950718 |
| R: AT, DE, ES, FR, GB, IT | | | | |
| US 5942659 | A | 19990824 | US 1997-776210 | 19970124 |
| PRIORITY APPLN. INFO.: | | | FR 1994-9272 A | 19940725 |
| | | | WO 1995-FR957 W | 19950718 |

AB A method of producing fatty acids or their derivs. from oil plants without the need for hydrolysis of oils with added exogenous **lipase** is described. Transgenic plants with a **lipase gene** under the control of a tissue-specific or inducible promoter are constructed.

The tissue-specific promoter drives expression outside the oil-accumulating tissues, the inducible promoter may be one that can be induced shortly before processing of the plant material. Upon processing the material, the breakdown of the plant tissue leads to mixing of the **lipase** and the lipids with a resulting hydrolysis and release of fatty acids or their derives from the lipids. A **gene** for a microbial **lipase**, e.g. from Candida, Rhizopus, or Pseudomonas, that is widely used in industrial fat hydrolysis may be used. The construction of a transgenic Brassica napus expressing a Rhizopus niveus **lipase cDNA** from a napin promoter is described.

L22 ANSWER 38 OF 59 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 96:886619 SCISEARCH
THE GENUINE ARTICLE: VV002
TITLE: Facile purification of highly active recombinant Staphylococcus hyicus **lipase** fragment and characterization of a putative lid region
AUTHOR: Chang R C; Chen J C; Shaw J F (Reprint)
CORPORATE SOURCE: ACAD SINICA, INST BOT, NANKANG 11529, TAIPEI, TAIWAN (Reprint); ACAD SINICA, INST BOT, NANKANG 11529, TAIPEI, TAIWAN; CHINA JR COLL MARINE TECHNOL, DEPT SEA FOOD TECHNOL, TAIPEI 111, TAIWAN; NATL TAIWAN OCEAN UNIV, INST MARINE BIOTECHNOL, CHILUNG 20224, TAIWAN
COUNTRY OF AUTHOR: TAIWAN
SOURCE: BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (21 NOV 1996) Vol. 228, No. 3, pp. 774-779.
Publisher: ACADEMIC PRESS INC JNL-COMP SUBSCRIPTIONS, 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495.
ISSN: 0006-291X.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 28
*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB A fragment of Staphylococcus hyicus **lipase gene** (Ala248 to Ala640) was inserted into plasmid pET20(b+). The resulting His-tagged recombinant protein (49 kDa) was overexpressed in Escherichia coli BL21(DE3) as an highly active **lipase** and was purified by nickel-coupled resin. Site-directed mutagenesis showed that in comparison with wild type enzyme, the L326F and L326A enzymes showed a 3.4 and 5 fold increase in the Km, respectively, but only a 44% and a 64% decrease in the kcat/Km, respectively, suggesting thar Leu326 of the putative lid participated in substrate-binding. (C) 1996 Academic Press, Inc.

L22 ANSWER 39 OF 59 CAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 14
ACCESSION NUMBER: 1996:212601 CAPLUS
DOCUMENT NUMBER: 124:287140
TITLE: Effect of the leader sequence on the expression of recombinant C. **rugosa lipase** by S. cerevisiae cells
AUTHOR(S): Fusetti, Fabrizia; Brocca, Stefania; Porro, Danilo; Lotti, Marina
CORPORATE SOURCE: Dipartimento Fisiologia Biochimica Generali, Universita Studi Milano, Milan, 20133, Italy
SOURCE: Biotechnology Letters (1996), 18(3), 281-6
CODEN: BILED3; ISSN: 0141-5492
PUBLISHER: Chapman and Hall
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The Candida **rugosa lipase** I **gene** was expressed in Saccharomyces cerevisiae. The recombinant **lipase** was efficiently synthesized only following the replacement of the enzyme endogenous leader sequence with the signal peptide of the Kluyveromyces lactis killer toxin. Amt. of accumulated **lipase** was 10-20 mg/L in batch culture and >1 g/L in a computer-controlled fed-batch fermn. system.

L22 ANSWER 40 OF 59 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1996:333944 CAPLUS
DOCUMENT NUMBER: 125:28683

TITLE: Protein engineering of a fungal **lipase**: towards expression of a recombinant Candida **rugosa lipase**
AUTHOR(S): Alberghina, Lilia; Lotti, Marina
CORPORATE SOURCE: Dipartimento Fisiologia e Biochimica Generali, Universita' degli Studi di Milano, Milan, 20133, Italy
SOURCE: NATO ASI Series, Series E: Applied Sciences (1996), 317(Engineering of/with Lipases), 219-228
CODEN: NAESDI; ISSN: 0168-132X
PUBLISHER: Kluwer
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB A review with 20 refs.

L22 ANSWER 41 OF 59 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 96:221118 SCISEARCH
THE GENUINE ARTICLE: TN944
TITLE: A STRUCTURAL BASIS FOR ENANTIOSELECTIVE INHIBITION OF CANDIDA-**RUGOSA LIPASE** BY LONG-CHAIN ALIPHATIC-ALCOHOLS
AUTHOR: HOLMQUIST M; HAEFFNER F; NORIN T; HULT K (Reprint)
CORPORATE SOURCE: ROYAL INST TECHNOL, DEPT BIOCHEM & BIOTECHNOL, TEKNIKRINGEN 34, S-10044 STOCKHOLM, SWEDEN (Reprint); ROYAL INST TECHNOL, DEPT CHEM, S-10044 STOCKHOLM, SWEDEN
COUNTRY OF AUTHOR: SWEDEN
SOURCE: PROTEIN SCIENCE, (JAN 1996) Vol. 5, No. 1, pp. 83-88. ISSN: 0961-8368.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 31
*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*
AB Molecular modeling showed that the enantiomers of heptyl 2-methyldecanoate are productively bound to the active site of Candida **rugosa lipase** in quite different conformations. The fast-reacting S-enantiomer may well occupy the previously identified acyl-binding tunnel in the active site of the **lipase**. By contrast, the slow-reacting R-enantiomer must be bound to the active site, leaving the tunnel empty to allow the formation of two catalytically essential hydrogen bonds between His 449 of the catalytic triad and the transition state of the catalyzed reaction. This information enables us to propose a molecular mechanism explaining how long-chain aliphatic alcohols act as enantioselective inhibitors of this **lipase** in the resolution of 2-methyldecanoic acid. Long-chain aliphatic alcohols may coordinate to the acyl-binding tunnel of the C. **rugosa lipase**, thereby selectively inhibiting the turnover of the fast-reacting S-enantiomer, thus resulting in a lowered enantioselectivity in the resolution.

L22 ANSWER 42 OF 59 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 97:5816 SCISEARCH
THE GENUINE ARTICLE: VX982
TITLE: Studying the active site pocket of Staphylococcus hyicus **lipase** by site-directed mutagenesis
AUTHOR: Chang R C; Chen J C; Shaw J F (Reprint)
CORPORATE SOURCE: NATL TAIWAN OCEAN UNIV, INST MARINE BIOTECHNOL, CHILUNG 20224, TAIWAN (Reprint); NATL TAIWAN OCEAN UNIV, INST MARINE BIOTECHNOL, CHILUNG 20224, TAIWAN; CHINA JR COLL MARINE TECHNOL, DEPT SEA FOOD TECHNOL, TAIPEI 111, TAIWAN; ACAD SINICA, INST BOT, TAIPEI 11529, TAIWAN
COUNTRY OF AUTHOR: TAIWAN
SOURCE: BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (4 DEC 1996) Vol. 229, No. 1, pp. 6-10.
Publisher: ACADEMIC PRESS INC JNL-COMP SUBSCRIPTIONS, 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495.
ISSN: 0006-291X.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English

REFERENCE COUNT: 33

*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB Site-directed mutagenesis of a previously constructed, recombinant Staphlococcus hyicus **lipase** (49 kDa) showed that Va1363 played a role in catalysis and substrate-binding. In comparison with wild type enzyme, the 64% and 89% decrease in the catalytic efficiency (kcat/K-m) of the V363N and V363A enzymes, respectively, were largely caused by a 3.5- and 5.5-fold increase in the substrate-binding affinity (K-m), respectively. In comparison with wild type enzyme, a G371A enzyme showed a 40% decrease in the k(m), suggesting that G371 was important for substrate-binding specificity. Site-directed mutagenesis of the active site Asp559 revealed that in comparison with wild type enzyme, a D559E enzyme exhibited a 47% decrease in the kcat/K-m, but a twofold increase in the K-m for p-nitrophenyl butyrate, suggesting that Asp-559, a component of the catalytic triad, was involved in substrate-specificity. (C) 1996 Academic Press, Inc.

L22 ANSWER 43 OF 59 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1995:645247 CAPLUS
DOCUMENT NUMBER: 123:29030
TITLE: **Lipase**-labelled probe
INVENTOR(S): Pittner, Fritz; Schalkhammer, Thomas; Ecker, Bernhard; Kynclova, Eva; Wakolbinger, Werner
PATENT ASSIGNEE(S): Boehringer Mannheim GmbH, Germany
SOURCE: PCT Int. Appl., 30 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: German
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 9510775 | A1 | 19950420 | WO 1994-EP3379 | 19941013 |
| W: AU, CA, JP, NZ, US | | | | |
| RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE | | | | |
| CA 2151731 | AA | 19950420 | CA 1994-2151731 | 19941013 |
| AU 9478557 | A1 | 19950504 | AU 1994-78557 | 19941013 |
| AU 671392 | B2 | 19960822 | | |
| JP 07509618 | T2 | 19951026 | JP 1994-511295 | 19941013 |
| EP 679257 | A1 | 19951102 | EP 1994-929543 | 19941013 |
| R: AT, CH, DE, ES, FR, GB, IT, LI | | | | |
| PRIORITY APPLN. INFO.: | | | AT 1993-2071 | 19931015 |
| | | | WO 1994-EP3379 | 19941013 |

AB To improve the thermal and chem. stability of an enzyme-labeled probe, a **lipase** that is preferably extd. from Candida **rugosa** and whose isoenzymes or structural analogs have at least 70% amino acid homol. and **lipase** activity is used as the enzyme.

L22 ANSWER 44 OF 59 MEDLINE on STN DUPLICATE 15
ACCESSION NUMBER: 96155626 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8575019
TITLE: Localization of **lipase genes** on Candida **rugosa** chromosomes.
AUTHOR: Brocca S; Grandori R; Breviario D; Lotti M
CORPORATE SOURCE: Dipartimento di Fisiologia e Biochimica Generali, Italia.
SOURCE: Current genetics, (1995 Oct) 28 (5) 454-7.
Journal code: 8004904. ISSN: 0172-8083.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199603
ENTRY DATE: Entered STN: 19960321
Last Updated on STN: 19960321
Entered Medline: 19960314

AB In the yeast Candida **rugosa** the **lipase** isozymes are encoded by a family of **genes**, five of which have been cloned and sequenced in our laboratory. In this paper we report on the identification and preliminary characterization of two new related sequences, thus extending this multigene family to seven members. The

total **DNA** content of Candida cells was estimated by laser flow-cytometry at about 20 Mb. Eight chromosomes with sizes ranging between 100 kb and 2.1 Mb, as determined by comparison with S. cerevisiae chromosomal bands, were resolved by pulsed-field gel electrophoresis. The **lipase**-encoding **genes** were localized on chromosome I, therefore suggesting that they have originated through multiple duplication events of an ancestral **gene**.

L22 ANSWER 45 OF 59 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1995:502091 CAPLUS
DOCUMENT NUMBER: 123:75827
TITLE: Oligonucleotide labeled **lipase** as a new sensitive hybridization probe and its use in bio-assays and biosensors
AUTHOR(S): Kynclova, Eva; Hartig, Andreas; Schalkhammer, Thomas
CORPORATE SOURCE: Inst. Biochem. Mol. Zellbiol., Univ. Vienna, Vienna, A-1030, Austria
SOURCE: Journal of Molecular Recognition (1995), 8(1/2), 139-45
CODEN: JMORE4; ISSN: 0952-3499
PUBLISHER: Wiley
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Radiolabeled polynucleotide probes have been employed extensively for the detection of complementary **nucleic acids** by specific hybridization. Within the last few years, various methods have been developed using enzyme-labeled probes to avoid unstable and hazardous isotopes. These assays, based on photometry, fluorescence, and chemiluminescence, have helped to overcome the use of radioactive probes. To increase the performance of a non-radioactive **DNA** detection system, the labeling enzyme should remain stable under hybridization conditions which allow the formation of a 15-25 bp long **DNA-DNA** duplex (Tm = 50-70.degree.). Therefore, the use of unstable phosphatase and peroxidase conjugates must be avoided due to the compn. of the hybridization mixt. and the high temp. By screening various hydrolytic enzymes to fit the special demands, fungal **lipases** turned out to be the most practical. They offer high sensitivity due to an extremely high turnover no., stability at room temp. for several years, thermostability under working conditions and an easy design of various chromogenic, fluorescent and electrochem. active substrates. Several types of silanized, oxidized and unmodified metal sensors and also std. microtiter plates modified with amino groups were used for the immobilization of oligonucleotides. A sandwich hybridization using the **lipase**-labeled oligonucleotide probe and a terminal immobilized capture **DNA** on a microtiter plate or sensor surface combined with a rapid hybridization in soln. simplifies and improves the performance of the **DNA** detection system.

L22 ANSWER 46 OF 59 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1995:291461 CAPLUS
DOCUMENT NUMBER: 122:283357
TITLE: Expression of recombinant Candida **rugosa lipase**
AUTHOR(S): Lotti, M.; Brocca, S.; Fusetti, F.; Alberghina, L.
CORPORATE SOURCE: Fisiologia e Biochimica Generali, Univ. di Milano, Milan, 20133, Italy
SOURCE: Mededelingen - Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen (Universiteit Gent) (1994), 59(4B), 2313-19
CODEN: MFLBER; ISSN: 1373-7503
DOCUMENT TYPE: Journal
LANGUAGE: English
AB In the asporogenic yeast Candida **rugosa lipase** is encoded by a family of **genes**. As a consequence, multiple **lipase** isoenzymes with similar but not identical mol. and catalytic properties are secreted in the culture medium. The **gene** LIP1, coding for the major **lipase** protein, was engineered by replacement of its leader sequence and expressed in S. cerevisiae under the control of an inducible promoter. Levels of expression as high as 20 mg/L of glycosylated intracellular protein were obtained.

L22 ANSWER 47 OF 59 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN

ACCESSION NUMBER: 94:656877 SCISEARCH
THE GENUINE ARTICLE: PK830
TITLE: COMPUTER MODELING OF SUBSTRATE-BINDING TO **LIPASES** FROM RHIZOMUCOR-MIEHEI, HUMICOLA-LANUGINOSA, AND CANDIDA-**RUGOSA**
AUTHOR: NORIN M; HAEFFNER F; ACHOUR A; NORIN T; HULT K (Reprint)
CORPORATE SOURCE: ROYAL INST TECHNOL, DEPT BIOCHEM & BIOTECHNOL, S-10044 STOCKHOLM, SWEDEN (Reprint); ROYAL INST TECHNOL, DEPT BIOCHEM & BIOTECHNOL, S-10044 STOCKHOLM, SWEDEN; ROYAL INST TECHNOL, DEPT CHEM, S-10044 STOCKHOLM, SWEDEN
COUNTRY OF AUTHOR: SWEDEN
SOURCE: PROTEIN SCIENCE, (SEP 1994) Vol. 3, No. 9, pp. 1493-1503. ISSN: 0961-8368.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 42

*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB The substrate-binding sites of the triacyl glyceride **lipases** from Rhizomucor miehei, Humicola lanuginosa, and Candida **rugosa** were studied by means of computer modeling methods. The space around the active site was mapped by different probes. These calculations suggested 2 separate regions within the binding site. One region showed high affinity for aliphatic groups, whereas the other region was hydrophilic. The aliphatic site should be a binding cavity for fatty acid chains. Water molecules are required for the hydrolysis of the acyl enzyme, but are probably not readily accessible in the hydrophobic interface, in which **lipases** are acting. Therefore, the hydrophilic site should be important for the hydrolytic activity of the enzyme.

**Lipases** from R. miehei and H. lanuginosa are excellent catalysts for enantioselective resolutions of many secondary alcohols. We used molecular mechanics and dynamics calculations of enzyme-substrate transition-state complexes, which provided information about molecular interactions important for the enantioselectivities of these reactions.

L22 ANSWER 48 OF 59 MEDLINE on STN DUPLICATE 16

ACCESSION NUMBER: 95094819 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8001575
TITLE: Pancreatic carboxylester **lipase** from Atlantic salmon (Salmo salar). **cDNA** sequence and computer-assisted modelling of tertiary structure.
AUTHOR: Gjellesvik D R; Lorens J B; Male R
CORPORATE SOURCE: Laboratory for Marine Molecular Biology, University of Bergen, Norway.
SOURCE: European journal of biochemistry / FEBS, (1994 Dec 1) 226 (2) 603-12.
Journal code: 0107600. ISSN: 0014-2956.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-L23929
ENTRY MONTH: 199501
ENTRY DATE: Entered STN: 19950215
Last Updated on STN: 20000303
Entered Medline: 19950126

AB We report the isolation and characterization of a 1795-bp **cDNA** fragment encoding Atlantic salmon pancreatic carboxylester **lipase** from salmon pancreas mRNA. The nearly full-length **cDNA** contained a 540-amino-acid open-reading frame, encompassing the mature protein (by similarity to mammalian carboxylester **lipase** enzymes). The salmon carboxylester **lipase** primary structure shared 58% identity with mammalian carboxylester **lipases**, lacking the proline-rich C-terminal repeats found in human and rat carboxylester **lipases**. Congruent with other esterase B type enzymes, the salmon carboxylester **lipase** contained a canonical serine-esterase catalytic triad motif consisting of serine, histidine and aspartic acid. Computer-assisted modelling of the tertiary structure for salmon carboxylester **lipase** was conducted using acetylcholine

esterase (Torpedo californica) as a template structure. The model, in conjunction with sequence comparisons and available enzymological data, has been used to locate putative bile-salt-binding and lipid-binding sites. The carboxylester **lipase** enzymes contain a unique, highly conserved insert region that may be associated with bile-salt binding. In the model structure, this region is located close to the active site, and contains a tyrosine residue with an adjacent carboxylester-**lipase**-conserved arginine. These traits have previously been predicted for the non-specific (regarding bile-salt hydroxylation) bile-salt-binding site in carboxylester **lipase** enzymes. At this site, a dihydroxy or trihydroxy bile-salt molecule may bind the tyrosine via hydrophobic interactions, the anionic bile-salt head group may bind the arginine, while hydrogen bonding between the bile-salt 12 alpha hydroxy group and an adjacent aspargine residue is possible. The model does not contain an active site 'lid' structure as found in other **lipases**. The carboxylester **lipase** structural homolog to the 'flap' of the **lipases** from Geotrichum candidum and Candida **rugosa** contains a carboxylester-**lipase**-conserved deletion that renders this region unable to cover the active site. Instead, the shortening of this loop leads to solvent exposure of the carboxylester **lipase** insert region, an additional indication of the functional importance of this region.

L22 ANSWER 49 OF 59 MEDLINE on STN DUPLICATE 17
ACCESSION NUMBER: 94302002 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8029208
TITLE: Variability within the Candida **rugosa** **lipases** family.
AUTHOR: Lotti M; Tramontano A; Longhi S; Fusetti F; Brocca S; Pizzi E; Alberghina L
CORPORATE SOURCE: Dipartimento Fisiologia e Biochimica Generali, Universita degli Studi di Milano, Italy.
SOURCE: Protein engineering, (1994 Apr) 7 (4) 531-5. Journal code: 8801484. ISSN: 0269-2139.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199408
ENTRY DATE: Entered STN: 19940818
Last Updated on STN: 19940818
Entered Medline: 19940808
AB Several fungi secrete **lipase** isozymes differing in biochemical properties and in some cases in substrate specificity. In the yeast Candida **rugosa**, a family of related **genes** encodes for multiple **lipase** proteins, highly homologous in sequence but partially different in the regions interacting with the substrate molecule. Analysis of these substitutions performed on the basis of multiple alignments and using a 3-D model of the enzyme, allows identification of a restricted number of amino acids possibly involved in substrate specificity of Candida **lipases**.

L22 ANSWER 50 OF 59 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 1993:503929 BIOSIS
DOCUMENT NUMBER: PREV199396127936
TITLE: Chemoenzymatic synthesis of pure enantiomeric 2-arylpropionic acids.
AUTHOR(S): Garcia, Mariano; Del Campo, Carmen; Llama, Emilio F.; Sanchez-Montero, Jose M.; Sinisterra, Jose V. [Reprint author]
CORPORATE SOURCE: Dep. Organic, Pharmaceutical Chemistry, Faculty Pharmacy, Univ. Complutense, 28040 Madrid, Spain
SOURCE: Tetrahedron, (1993) Vol. 49, No. 37, pp. 8433-8440. CODEN: TETRAB. ISSN: 0040-4020.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 5 Nov 1993
Last Updated on STN: 6 Nov 1993
AB A new chemoenzymatic procedure to obtain pure enantiomeric 2-arylpropionic acids is described. The one pot synthesis of (+-)-2-arylpropionic acids

is carried out by addition of dichlorocarbene to the C dbd O bond of arylmethylketones and hydrogenolysis of the addition product. The racemic mixture is resolved by enantiospecific hydrolysis of the racemic ethyl esters using native **lipase** from Candida **rugosa**. The good yields, the accessibility of the starting arylmethylketones and the stereospecificity of the enzymatic hydrolysis make the process interesting in order to obtain the same non steroidal antiinflammatory drugs such as Ibuprofen or Naproxen.

L22 ANSWER 51 OF 59 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1993:553043 CAPLUS
DOCUMENT NUMBER: 119:153043
TITLE: Structure of a family of **lipase**-encoding **genes** from Candida cylindracea
AUTHOR(S): Fusetti, F.; Lotti, M.; Brocca, S.; Longhi, S.; Alberghina, L.
CORPORATE SOURCE: Dip. Fisiol. Biochim. Gen., Univ. Milano, Milan, I-20133, Italy
SOURCE: Mededelingen van de Faculteit Landbouwwetenschappen, Universiteit Gent (1992), 57(4b), 2045-52
CODEN: MFLRA3; ISSN: 0368-9697
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Five **genes** coding for **lipase** have been isolated from the yeast Candida cylindracea and their nucleotide sequences have been detd. The deduced amino acid sequences correspond to 534 residues mature proteins, each preceded by signal sequences for secretion. **Lipase** proteins, although related by high sequence homol., differ in their isoelec. points and in glycosylation sites. Based on the high sequence homol. with the Geotrichum candidum **lipases** and with Torpedo californica acetylchlorinesterase, a structural model of C. cylindracea **lipase** was inferred. The authors propose that C. cylindracea **lipases** have a Ser-Glu-His catalytic triad, with the active serine surrounded by a Y/FGESAG consensus sequence and a structural organization very similar to that of both ref. enzymes.

L22 ANSWER 52 OF 59 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1993:403830 CAPLUS
DOCUMENT NUMBER: 119:3830
TITLE: Cloning and nucleotide sequences of two **lipase** **genes** from Candida cylindracea
AUTHOR(S): Longhi, Sonia; Fusetti, Fabrizia; Grandori, Rita; Lotti, Marina; Vanoni, Marco; Alberghina, Lilia
CORPORATE SOURCE: Dip. Fisiol., Univ. Studi Milano, Milan, Italy
SOURCE: Biochimica et Biophysica Acta (1992), 1131(2), 227-32
CODEN: BBACAQ; ISSN: 0006-3002
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Two **lipase**-encoding **genes** (LIP1 and LIP2) were isolated from a SacI genomic library of the yeast C. cylindracea and their nucleotide sequences were detd. Comparison with the sequence of a **cDNA** ruled out the presence of introns in the 2 **genes**. Both ORFs encode for mature proteins of 534 residues with putative signal peptides of 15 and 14 amino acids, resp. When compared with other **lipase** sequences, the 2 C. cylindracea **lipases** showed homol. only with the Geotrichum candidum **lipase**, whereas they shared a significant similarity with several esterases.

L22 ANSWER 53 OF 59 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1993:35111 CAPLUS
DOCUMENT NUMBER: 118:35111
TITLE: Homology-derived three-dimensional structure prediction of Candida cylindracea **lipase**
AUTHOR(S): Longhi, Sonia; Lotti, Marina; Fusetti, Fabrizia; Pizzi, Elisabetta; Tramontano, Anna; Alberghina, Lilia
CORPORATE SOURCE: Dip. di Fisiol. e Biochim. Gen., Sez. di Biochim. Comp., Univ. degli Stud. di Milano, Milan, Italy
SOURCE: Biochimica et Biophysica Acta (1992), 1165(1), 129-33
CODEN: BBACAQ; ISSN: 0006-3002
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A structural model of C. cylindracea **lipase** (CCL) is proposed based on the reported x-ray structure of the highly homologous Geotrichum candidum **lipase** (GCL). The network of interactions around the active site, the salt and disulfide bridge pattern is conserved in the proposed structure. Functional, structural and evolutionary aspects of the peculiar usage of CTG codons by C. cylindracea ATCC 14830 are discussed.

L22 ANSWER 54 OF 59 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1992:167539 CAPLUS
DOCUMENT NUMBER: 116:167539
TITLE: Molecular cloning of a **lipase** and of a **lipase**-related **gene** from Candida cylindracea
AUTHOR(S): Alberghina, Lilia; Grandori, Rita; Longhi, Sonia; Lotti, Marina; Fusetti, Fabrizia; Vanoni, Marco
CORPORATE SOURCE: Dip. Fisiol. Biochim. Gen., Univ. Milano, Milan, 20133, Italy
SOURCE: GBF Monographs (1991), 16(Lipases), 231-5
CODEN: GBMOEB; ISSN: 0930-4320
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The screening of genomic libraries from C. cylindracea with synthetic oligonucleotides has led to the isolation of 2 **lipase**-related sequences. They have been characterized by restriction mapping and partially sequenced. One of them contains a **lipase**-coding sequence.

L22 ANSWER 55 OF 59 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1993:140913 CAPLUS
DOCUMENT NUMBER: 118:140913
TITLE: The structure of **lipase genes** and pseudogenes of Candida cylindracea
AUTHOR(S): Kawaguchi, Yoshiyuki; Honda, Hiroshi
CORPORATE SOURCE: Tokyo Res. Lab., Meito Sangyo Co. Ltd., Hachioji, 192, Japan
SOURCE: GBF Monographs (1991), 16(Lipases), 221-30
CODEN: GBMOEB; ISSN: 0930-4320
DOCUMENT TYPE: Journal
LANGUAGE: English
AB C. cylindracea produces at least two kinds of extracellular **lipase**, **lipase** I and **lipase** II. From the mutant which produces a large amt. of **lipase** I and a negligible amt. of **lipase** II, the authors obtained the **lipase** I **cDNAs** and genomic **genes**. **Lipase** I **genes** were classified into several types by sequencing and restriction anal. **Lipase** I proteins were thought to be encoded by several homologous **genes**. **Lipase** I was homologous with Geotrichum candidum **lipases**. Furthermore, pseudogenes were found with more than 90% homol. with **lipase** I **cDNA**. Although the amino acid compn. of purified **lipase** I was consistent with that deduced from the **cDNA** sequence, significant discrepancies were evident in the contents of leucine and serine. These discrepancies are due to the different genetic code used by C. cylindracea, in which the universal codon for leucine, CUG, is used to code for serine.

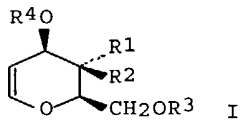
L22 ANSWER 56 OF 59 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 1993:421889 BIOSIS
DOCUMENT NUMBER: PREV199345069514
TITLE: Crystallization and characterization of Candida **rugosa lipase**.
AUTHOR(S): Rubin, Byron; Jamison, Penny; Harrison, David
CORPORATE SOURCE: Eastman Kodak Co., Rochester, NY 14650, USA
SOURCE: Alberghina, L. [Editor]; Schmid, R. D. [Editor]; Verger, R. [Editor]. GBF Monographs, (1991) pp. 63-66. GBF Monographs; Lipases: Structure, mechanisms and genetic engineering. Publisher: VCH Verlagsgesellschaft mbH, Postfach 10 11 61, Boschstrasse 12, D-6940 Weinheim, Germany; VCH Publishers, Inc., Suite 909, 220 East 23rd Street, New York, New York

10010, USA. Series: GBF Monographs.
Meeting Info.: CEC-GBF (Commission of the European Communities-Gesellschaft fuer Biotechnologische Forschung; (Society for Biotechnological Research) International Workshop. Braunschweig, Germany. September 13-15, 1990.
ISSN: 0930-4320. ISBN: 3-527-28332-3, 1-56081-165-X.
DOCUMENT TYPE: Article
Conference; (Meeting)
LANGUAGE: English
ENTRY DATE: Entered STN: 15 Sep 1993
Last Updated on STN: 15 Sep 1993

L22 ANSWER 57 OF 59 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 91:5398 SCISEARCH
THE GENUINE ARTICLE: EN927
TITLE: PURIFICATION AND SPECIFICITY OF **LIPASES** FROM GEOTRICHUM-CANDIDUM
AUTHOR: BAILLARGEON M W (Reprint)
CORPORATE SOURCE: USDA ARS, EASTERN REG RES CTR, 600 E MERMAID LANE, PHILADELPHIA, PA, 19118 (Reprint)
COUNTRY OF AUTHOR: USA
SOURCE: LIPIDS, (1990) Vol. 25, No. 12, pp. 841-848.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE; AGRI
LANGUAGE: ENGLISH
REFERENCE COUNT: 31
*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB A crude, commercial Geotrichum candidum **lipase** (EC 3.1.1.3) preparation (Amano GC-20) was purified by hydrophobic interaction chromatography on Octyl Sepharose. The purified enzyme is a microheterogeneous glycoprotein containing isozymes varying in molecular weight, pI and specificity. It consists of 64, 62 and 59 kDa species as determined by denaturing polyacrylamide gel electrophoresis. Five isozymes (pI 4.40, 4.47, 4.58, 4.67 and 4.72) are detected by isoelectric focusing using both silver and activity stains. Chromatofocusing was used to separate the isozymes according to pI. Although all the isozymes are specific for oleate vs stearate esters, one isozyme (pI 4.72) is also specific for oleate vs palmitate. The number of isozymes is reduced to two (pI 4.67 and 4.72) after carbohydrate removal using endoglycosidase F/N-glycosidase. These isozymes may be products of two **lipase genes**.

L22 ANSWER 58 OF 59 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1989:135586 CAPLUS
DOCUMENT NUMBER: 110:135586
TITLE: Enzymic synthesis of selectively protected glycals
AUTHOR(S): Holla, E. Wolfgang
CORPORATE SOURCE: Hoechst A.-G., Frankfurt/Main, D-6280/80, Fed. Rep. Ger.
SOURCE: Angewandte Chemie (1989), 101(2), 222-3
CODEN: ANCEAD; ISSN: 0044-8249
DOCUMENT TYPE: Journal
LANGUAGE: German
OTHER SOURCE(S): CASREACT 110:135586
GI



I

AB Glycols I (R1 = OH, R2-R4 = H) were acylated by MeCO2CH:CH2 in the

presence of Candida cylindracea **lipase** to **gene** I (R1 = OH, R2 = R4 = H, R3 = Ac) while in the presence of Pseudomonas fluorescens **lipase** P I (R1 = OH, R2 = R3 = R4 = Ac) resulted. Acylation of I (R1 = OH, R2 = R4 = H, R3 = Ac, Bz) by PhCO2CH:CH2 in the presence of **lipase** P gave I (R1 = OH, R2 = H, R3 = Ac, Bz, R4 = ClCH2CO), resp.

L22 ANSWER 59 OF 59 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1990:31430 CAPLUS
DOCUMENT NUMBER: 112:31430
TITLE: The codon CUG is read as serine in an asporogenic yeast Candida cylindracea
AUTHOR(S): Kawaguchi, Yoshiyuki; Honda, Hiroshi; Taniguchi-Morimura, Junko; Iwasaki, Shinjiro
CORPORATE SOURCE: Tokyo Res. Lab., Meito Sangyo Co. Ltd., Hachioji, 192, Japan
SOURCE: Nature (London, United Kingdom) (1989), 341(6238), 164-6
CODEN: NATUAS; ISSN: 0028-0836
DOCUMENT TYPE: Journal
LANGUAGE: English
AB An example of divergence from the universal genetic code is reported for the asporogenous yeast C. cylindracea. The nucleotide and encoded peptide sequences of a **cDNA** for **lipase** I was detd. The deduced amino acid sequence presumes that the universal codon for leucine, CUG, encodes serine. This conclusion is based on the observations that: (1) the amino-acid compn. and the partial amino-acid sequences of an extracellular **lipase** from this yeast agreed with those deduced from the **cDNA** if CUG was assumed to specify serine; and (2) serine, but not leucine, was incorporated into a polypeptide in a cell-free translation system from this yeast in the presence of a synthetic CUG oligomer.

=> file reg
=> s candida rugosa/bi
L24 51 CANDIDA RUGOSA/BI

=> d 51

L24 ANSWER 51 OF 51 REGISTRY COPYRIGHT 2004 ACS on STN
RN 124566-17-2 REGISTRY
CN Lipase, triacylglycerol (Candida cylindracea clone .lambda.CL115 reduced) (9CI) (CA INDEX NAME)
OTHER NAMES:
CN **Lipase IA (Candida rugosa reduced)**
FS PROTEIN SEQUENCE
MF Unspecified
CI MAN
SR CA
LC STN Files: CA, CAPLUS, CASREACT
DT.CA CAplus document type: Journal
RL.NP Roles from non-patents: BIOL (Biological study); PRP (Properties); RACT (Reactant or reagent); USES (Uses)

=> log y